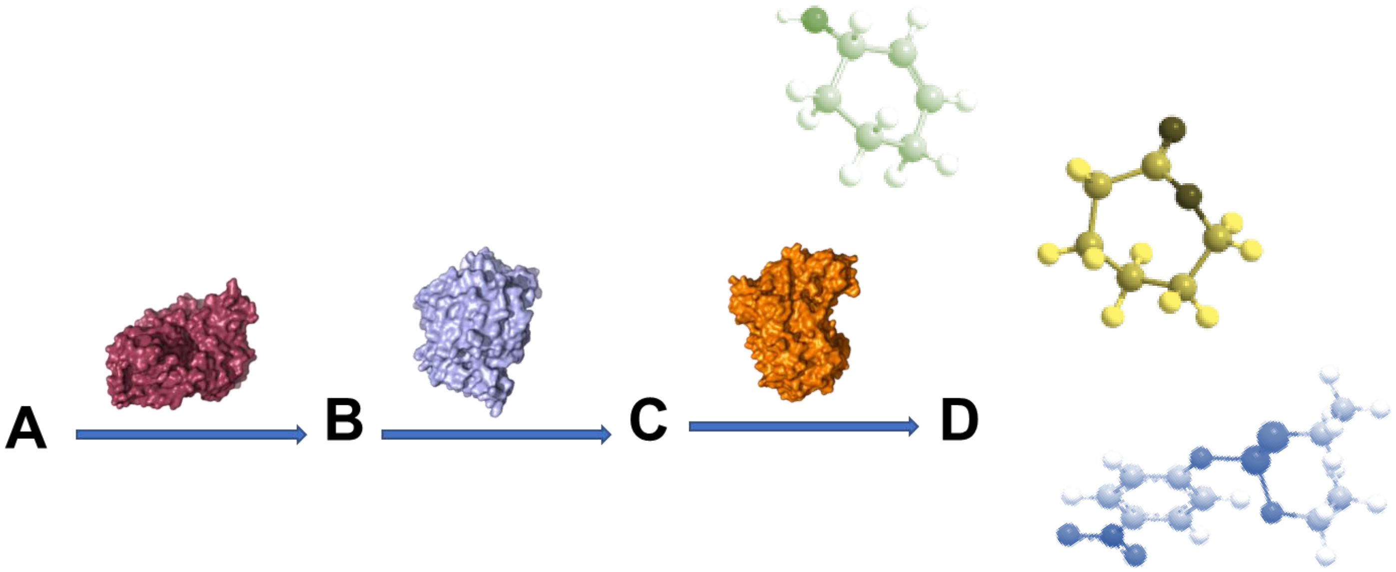


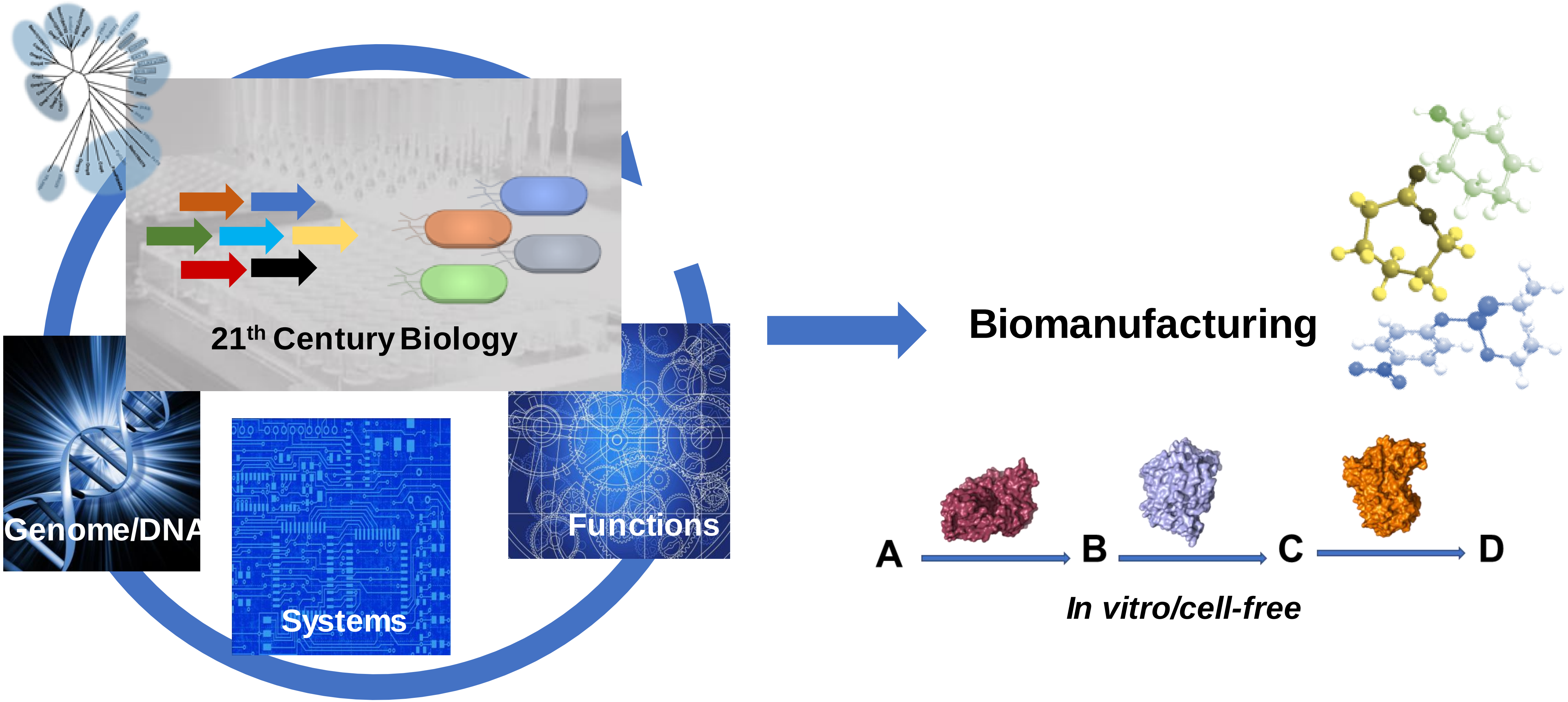
Cell-free reaction platforms for multi-enzyme biocatalysis
Challenges & Opportunities

Claudia Schmidt-Dannert

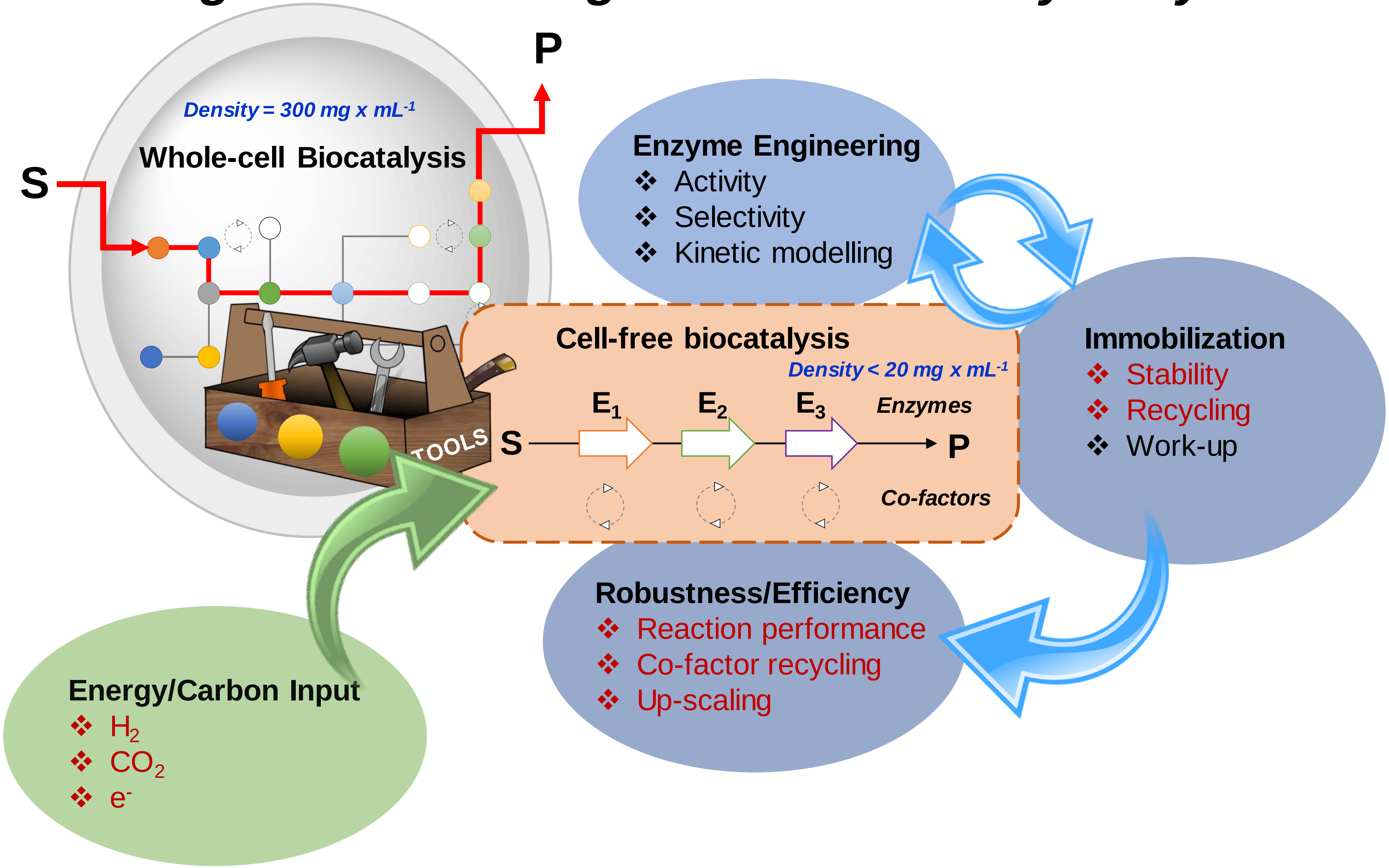
Dept. Biochemistry, Molecular Biology & Biophysics
BioTechnology Institute

University of Minnesota

Opportunities for the design of new biocatalytic systems



Challenges for the design of new biocatalytic systems

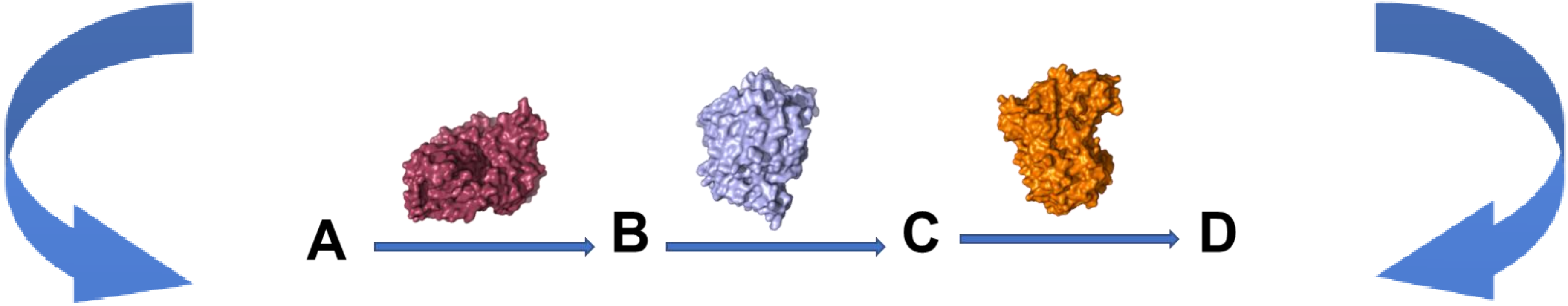


Adapted from: Schmidt-Danert, C. & Lopez-Gallego, F (2019) Curr Opin. Chem Biol. 49:97-104

*Fourth wave of biocatalysis**

New enzyme classes for industrial biocatalysis

Rapid design of tailored enzyme reactions
(Sequence/structure databases, HT-design)



<u>Well-developed</u>
Amine synthesis
Transaminases
Ketoreductase/Alcohol DH
Nitrilases

<u>Emerging</u>
Imine reductases
C-H Oxidations
Aldolases

Large repertoire of reactions for the design of long enzyme cascades

New approaches for reaction and process design

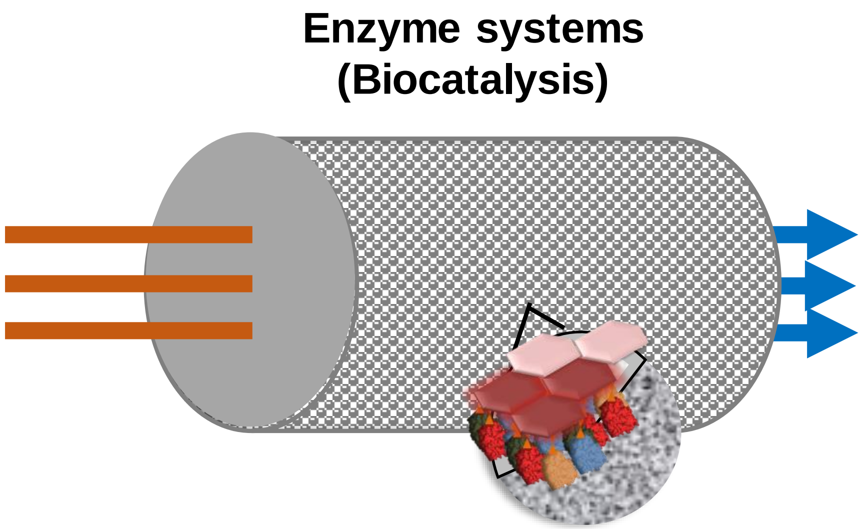
Pharmaceuticals & Bulk Chemicals

Schrittwieser, J.H. et al. (2018) Chem Rev.118:270-348

***Bornscheuer, U.T. (2018) Philos Trans A Math Phys Eng Sci. 376(2110)**

Merging synthetic biology and materials sciences

Design of robust self-organized systems for biocatalysis



Biomanufacturing

Genetically programmable

De novo production

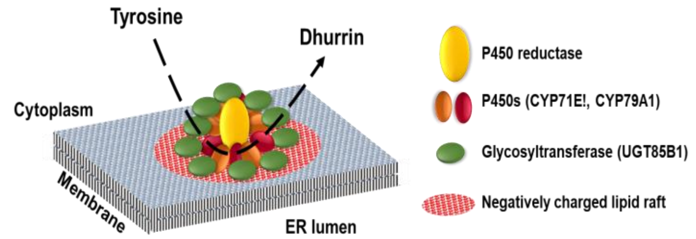
Self-organization

Biomineralization

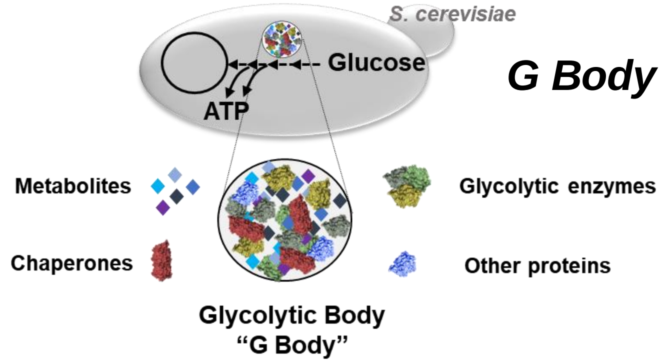
Self-organization in nature

Assembly of enzymes inside cells

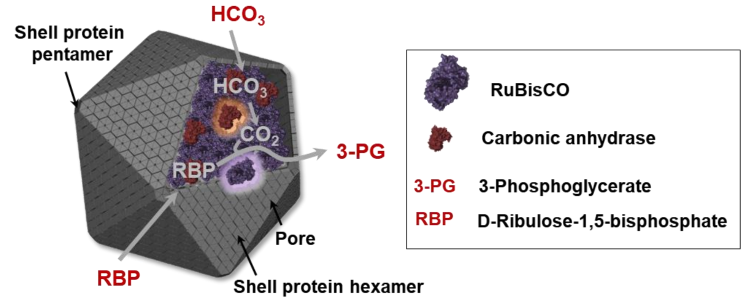
Metabolon



G Body



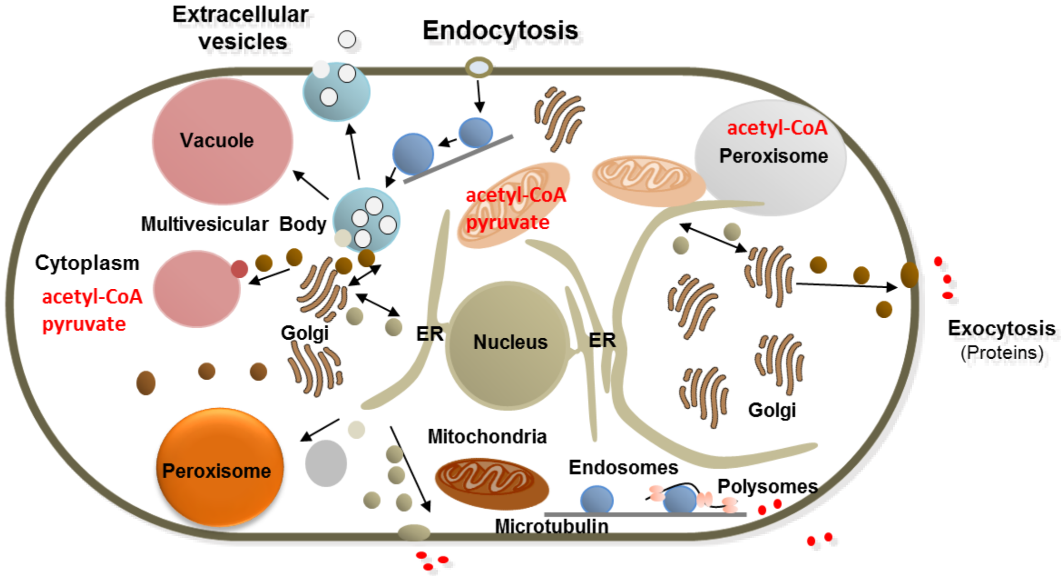
Carboxysome



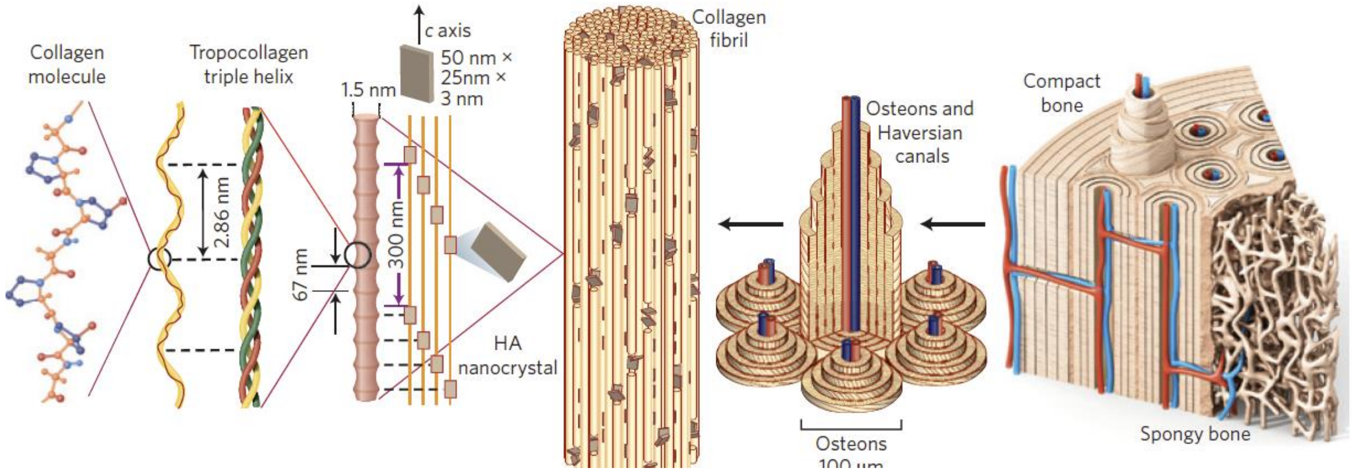
Bassard group: Science. 2016 354:890-893, Methods Enzymol. 2019 617:1-27

From: Schmidt-Dannert, Lopez-Gallego Curr. Opin. Chem. Biol., In Press

Compartmentalization inside cells



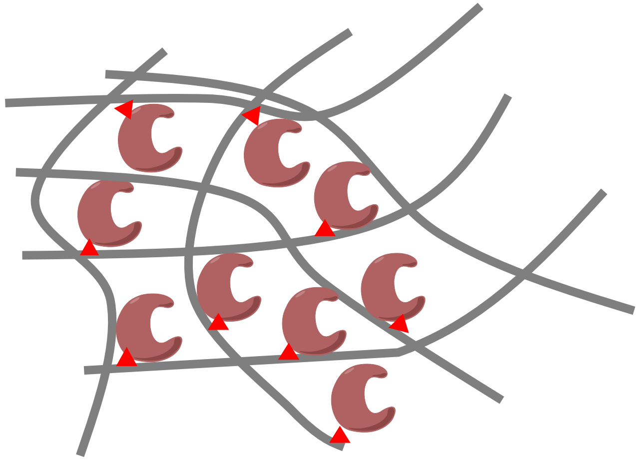
Protein-templated higher ordered structures - Bone



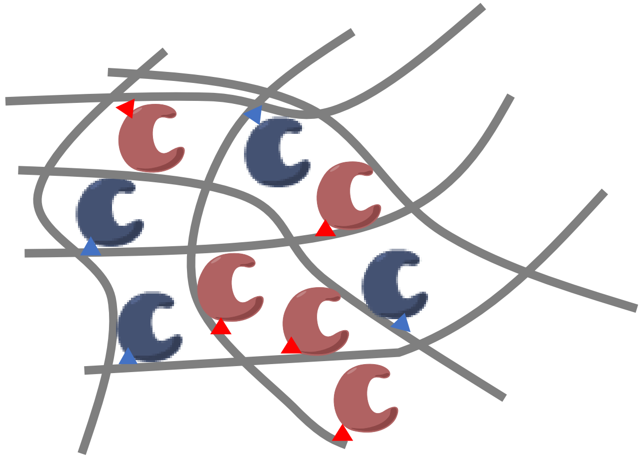
From: Nature Mat 2015 14:23-36

Enzyme immobilization

Enzyme stability & activity



One-pot multi-enzyme catalysis



Challenges → *Enzyme compatible immobilization chemistries*
→ *Operation of complex cascade reactions*

Manufacturing of enzyme system

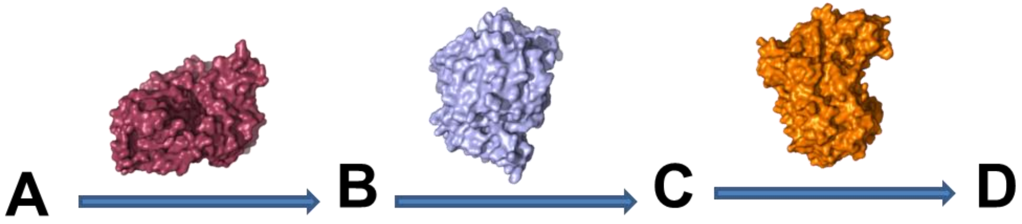
Production → Isolation → Immobilization → Operation → Reuse

Scale-able | **Cost-efficient** | **Cheap, mild, versatile** | **Robust & Efficient** (High TOF & TON)

Protein-based materials

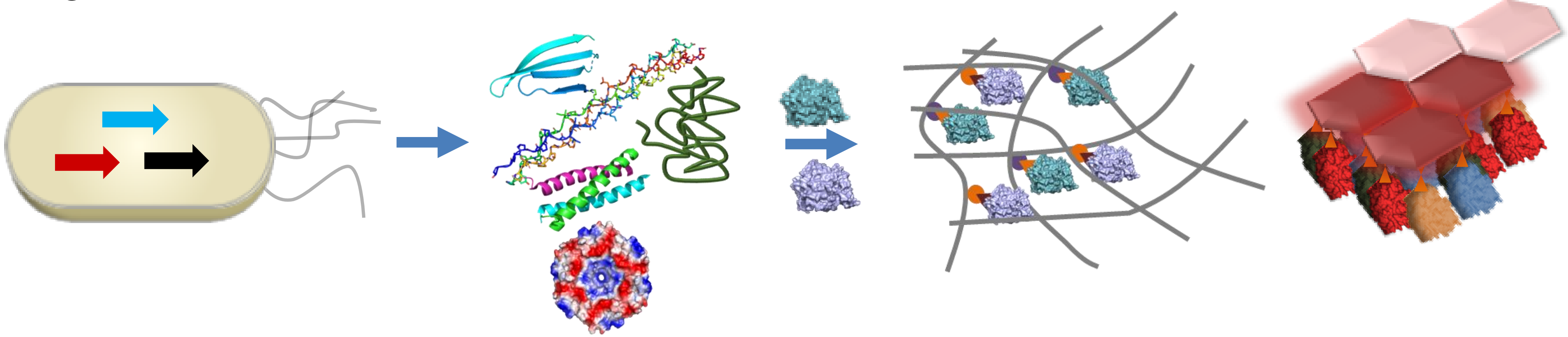
Development of structurally ordered biocomposite material with configurable (genetically programmable) material properties and embedded **biological capabilities**.

Biocatalytic cascade

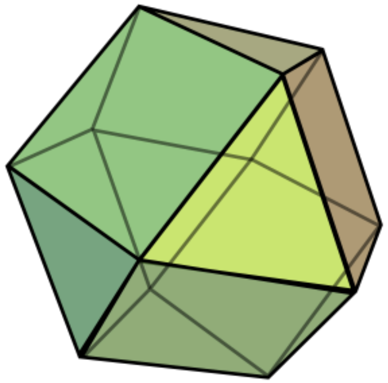


Configuration - Fabrication

Scaffolding - Immobilization - Operation

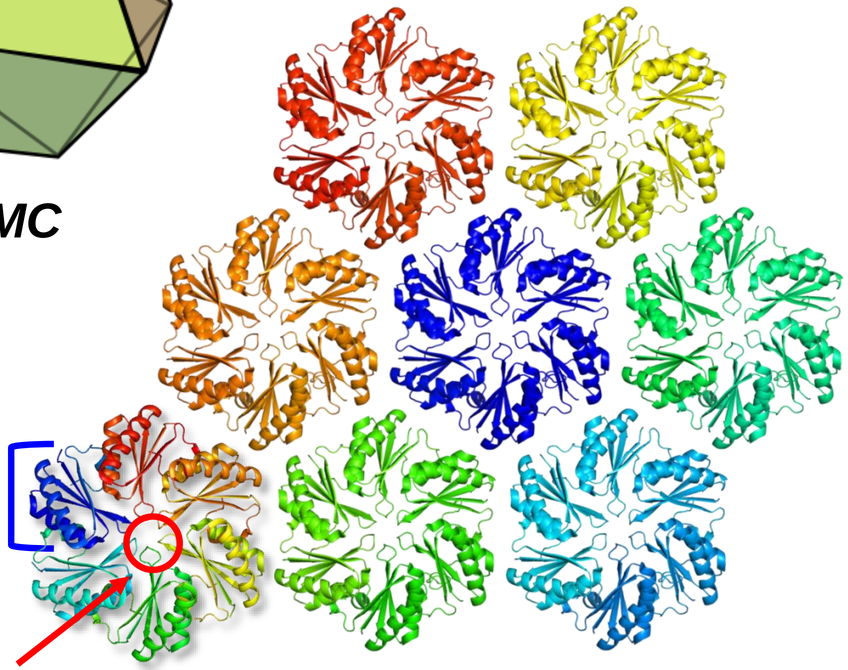


Bacterial MicroCompartment shell protein as scaffold building block



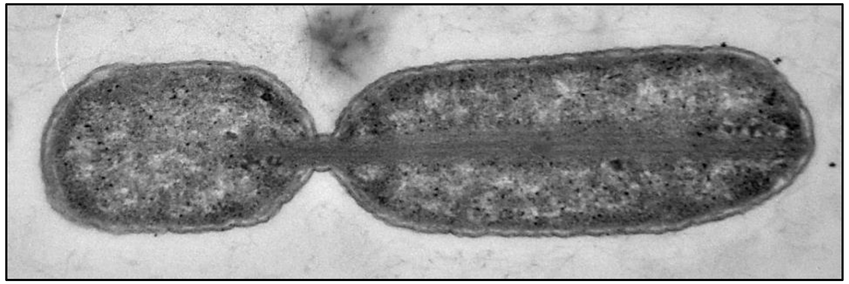
Eut BMC

Monomer
~11 kDa

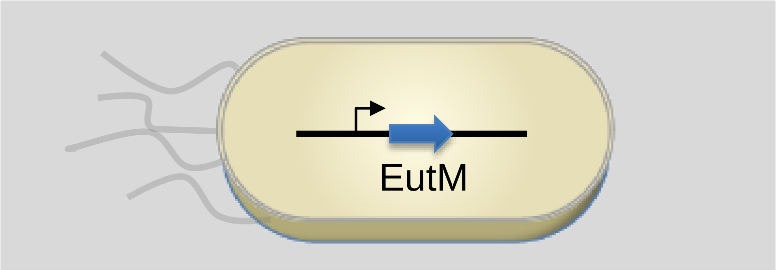


Pore

EutM self-assembles as a hexameric crystal lattice

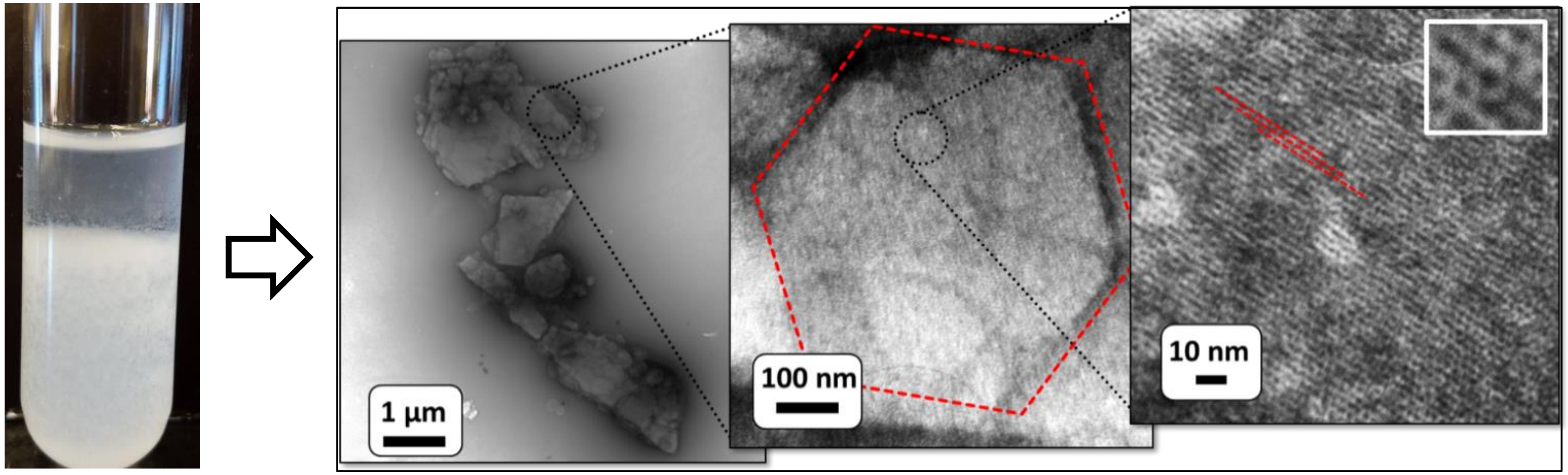


EutM expression in *E. coli* – protein scaffolds self-assemble *in vivo*



Choudhary et al. (2012) PLoS One 7(3): e33342; Quin et al. (2016) Appl Microbiol Biotechnol 100(21): 9187-9200; Held et al. (2016) Sci Rep 6: 24359

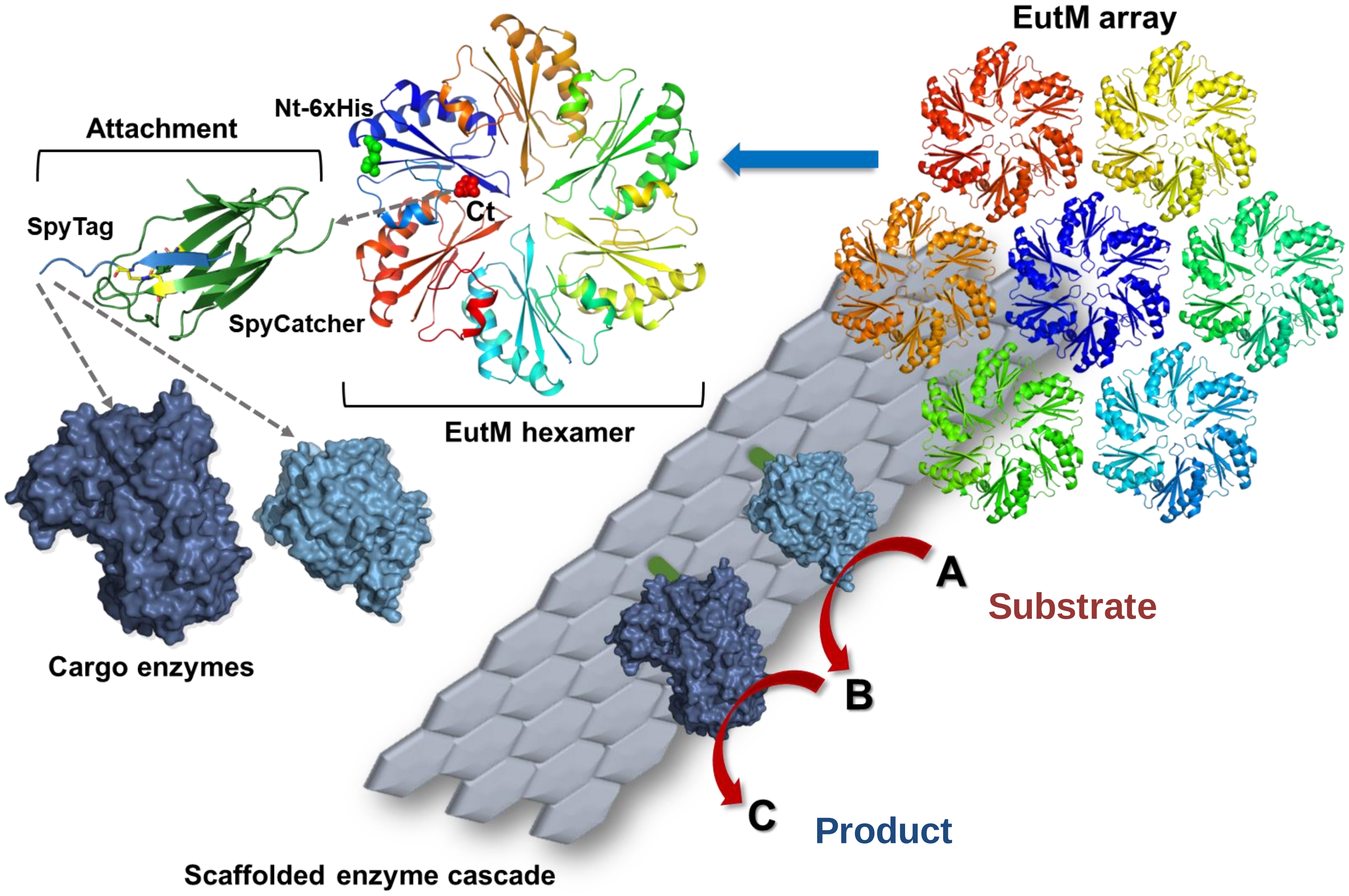
Self-assembling into large scaffolds



EutM self-assembles *in vitro* as hexameric tiles and arrays

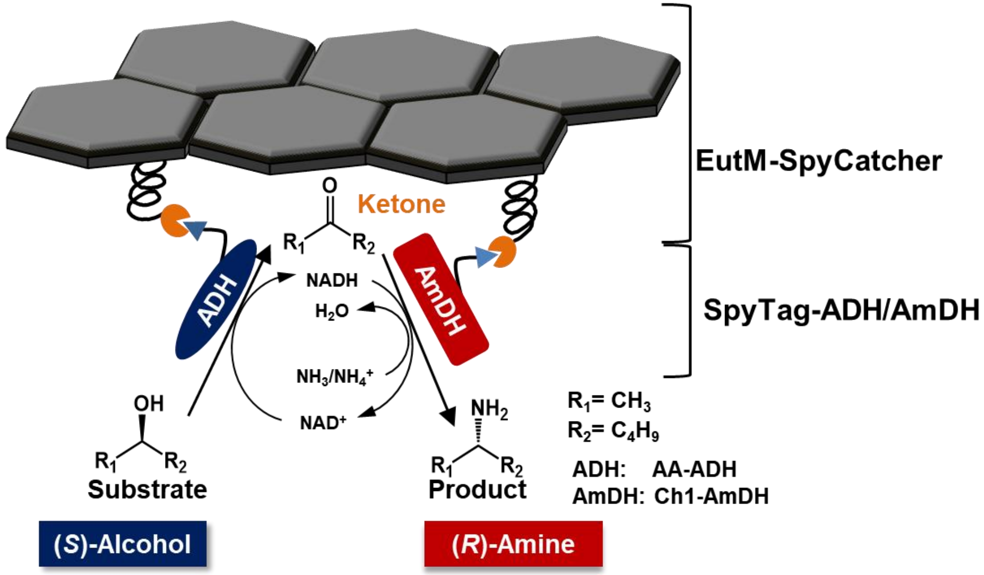
ACS Catalysis (2018) 8:5611-20

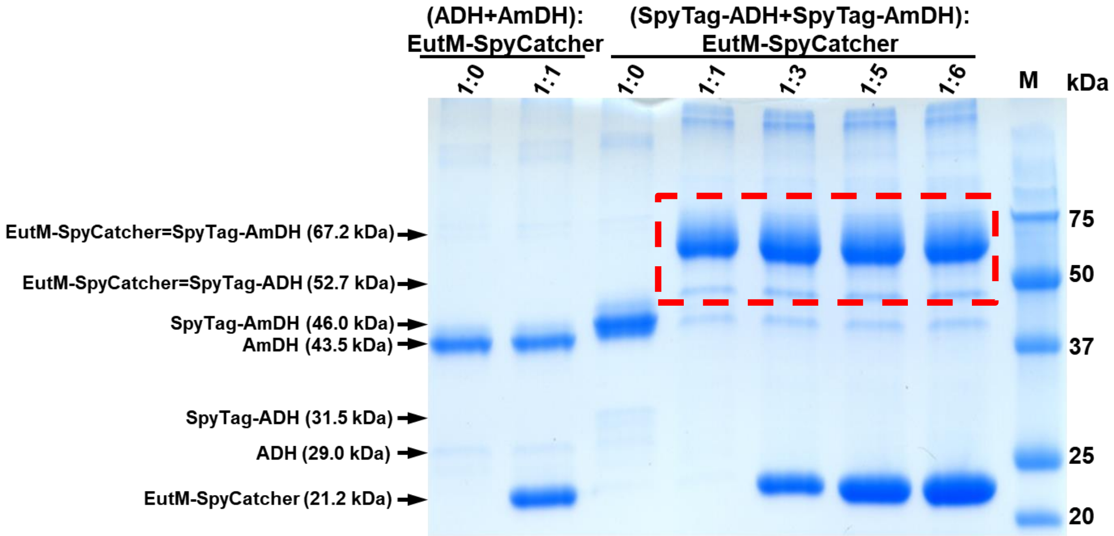
Design of scaffolds for biocatalysis

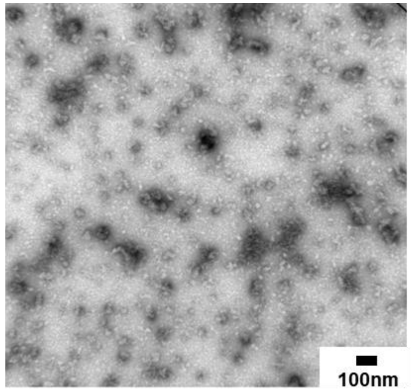


ACS Catalysis (2018) 8:5611-20

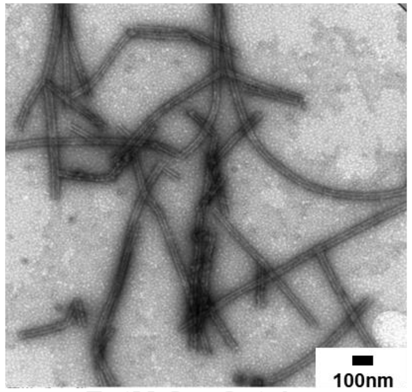
Co-immobilization of enzyme cascade



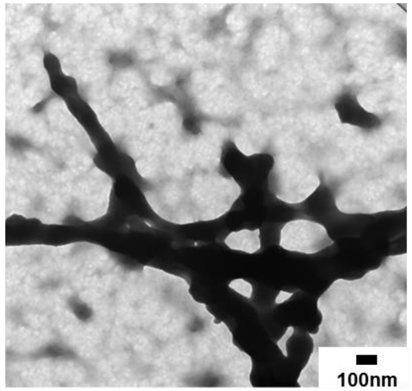




SpyTag-ADH + SpyTag-AmDH



EutM-SpyCatcher

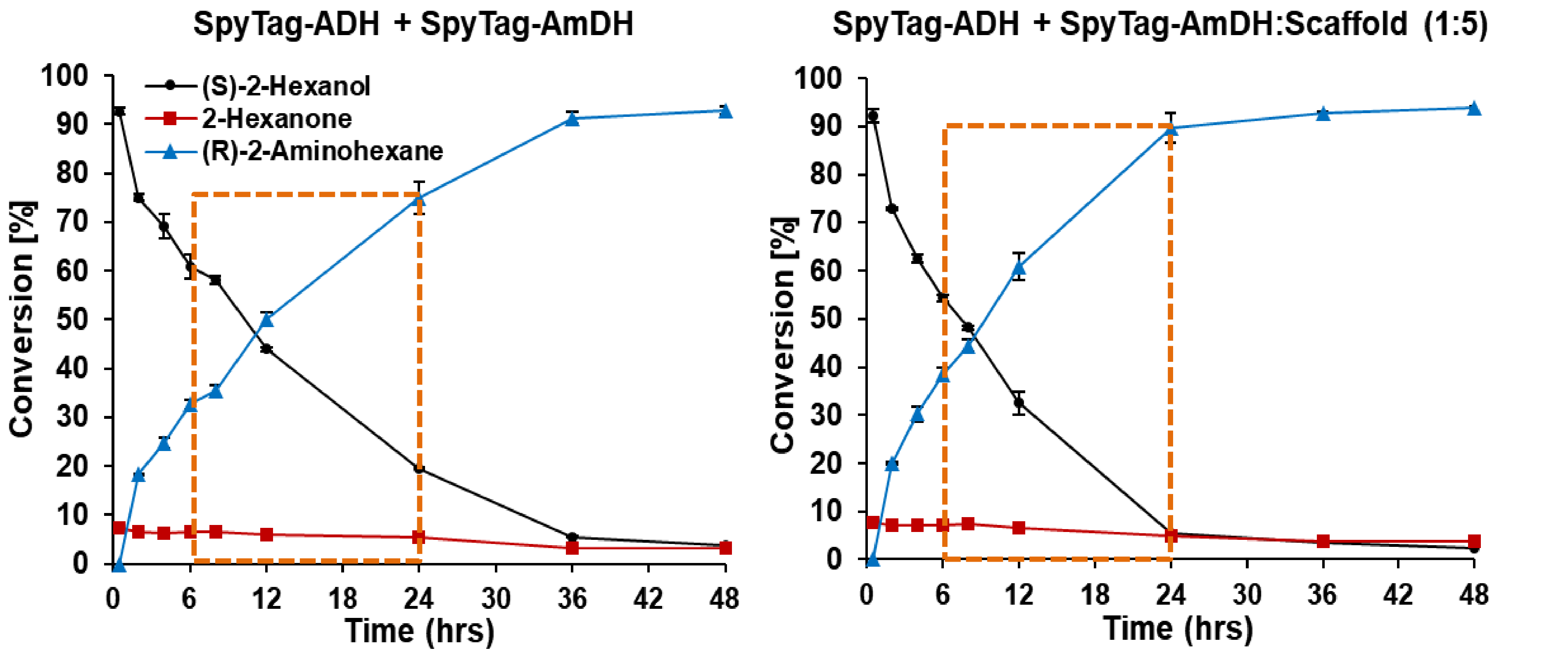


EutM-SpyCatcher + SpyTag-ADH + SpyTag-AmDH

2 M ammonium chloride buffer pH 8.7
(1:5 molar ratio enzymes:scaffold)
6 μM SpyTag-ADH
150 μM SpyTag-AmDH
780 μM EutM-SpyCatcher

ACS Catalysis (2018) 8:5611-20

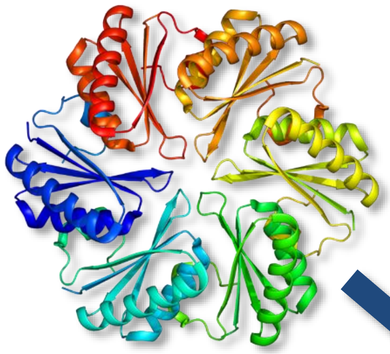
Optimization and testing of enzyme cascade



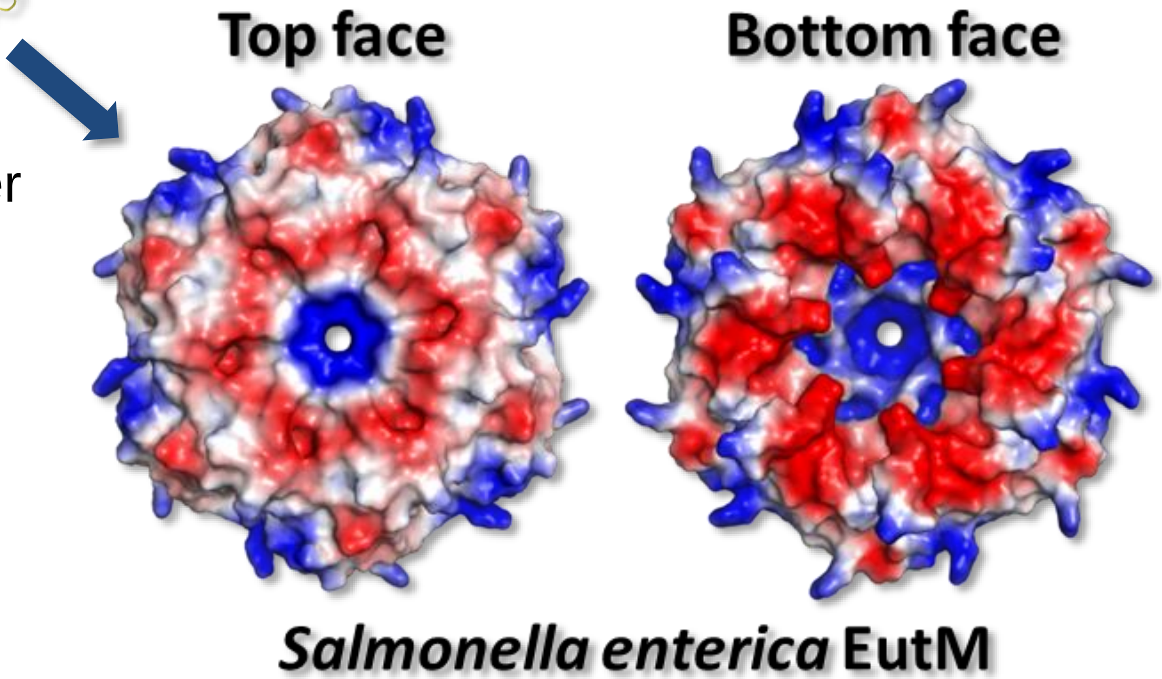
Scaffolds shorten reaction time to reach 90% conversion of 20 mM (S)-2-hexanol to (R)-2-aminohexane in 24 hrs with >99% ee.

ACS Catalysis (2018) 8:5611-20

Scaffolds define electrostatics & architecture of materials



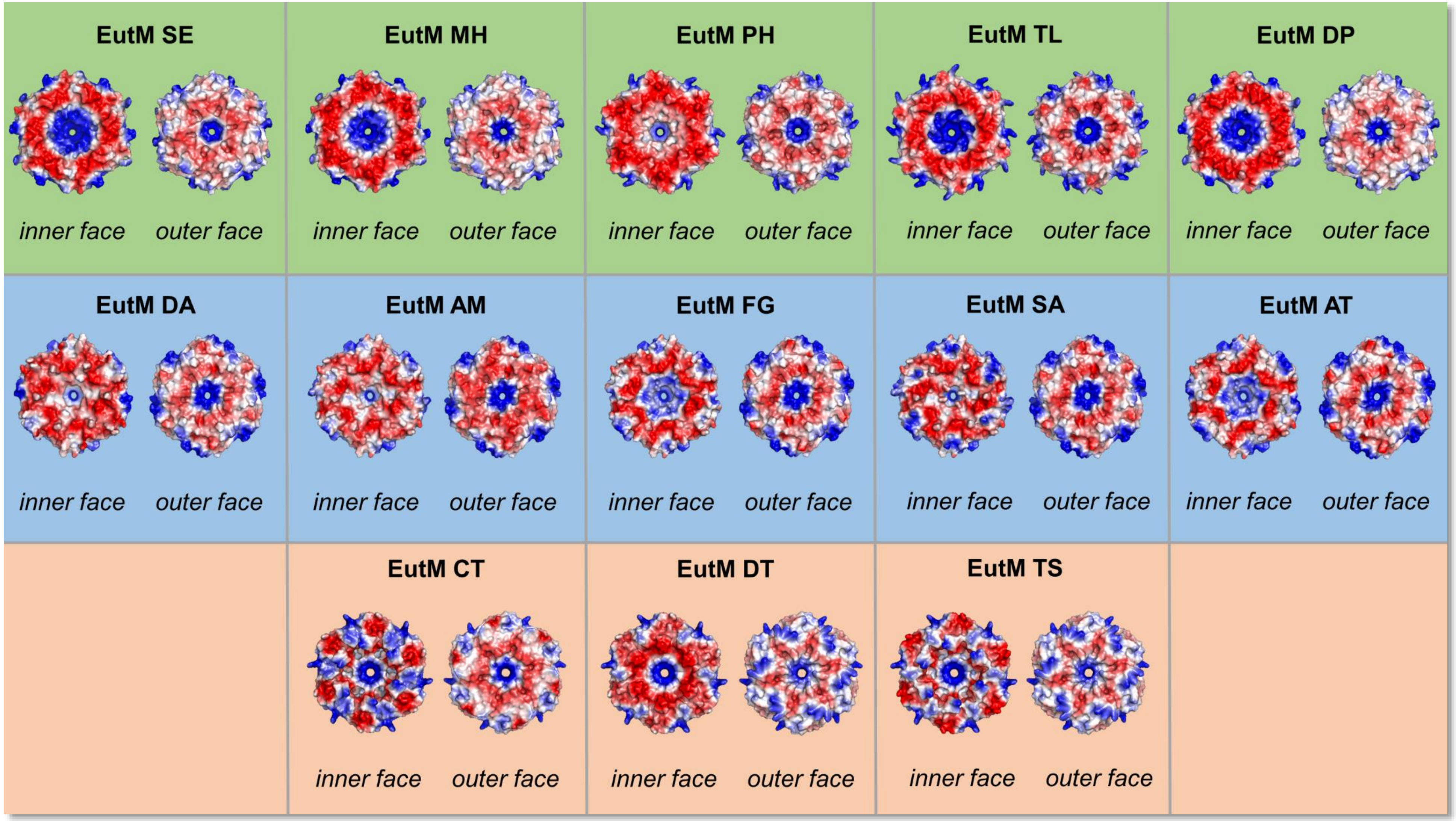
EutM hexamer



Surface electrostatic rendering

Zhang, Tsitkov and Hess (2016) Nat Commun. 7:13982
Zhang and Hess (2019) ACS Cent Sci. 5:939-948.

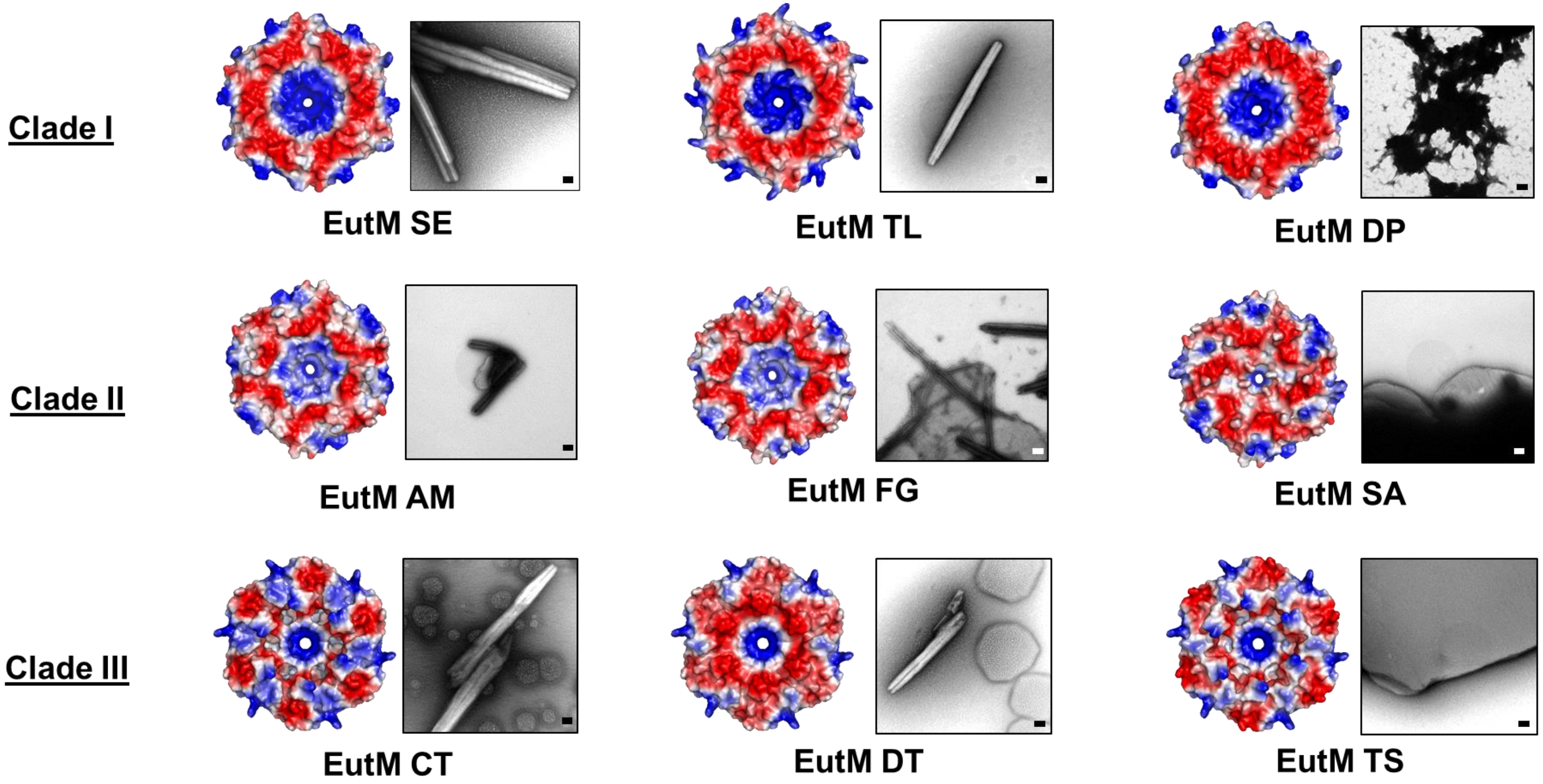
Expanding scaffold diversity



Appl. Microbiol. Biotechnol. (2018) 102:8373-8388

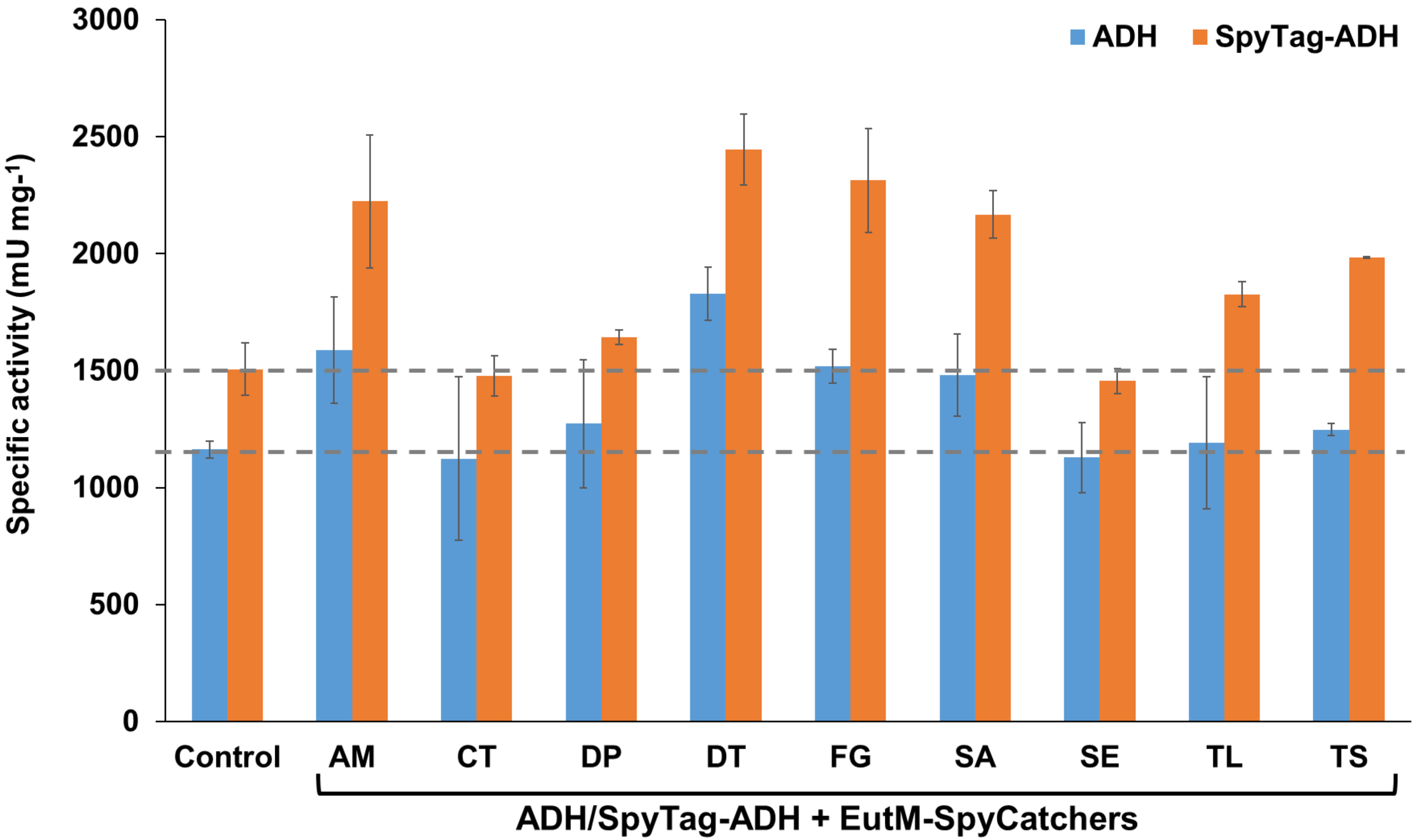
EutM-SpyCatcher building blocks

8 EutM homologs selected



ACS Synth Biol (2019) 8:1867-1876.

Enzyme immobilization on EutM-SpyCatcher scaffolds

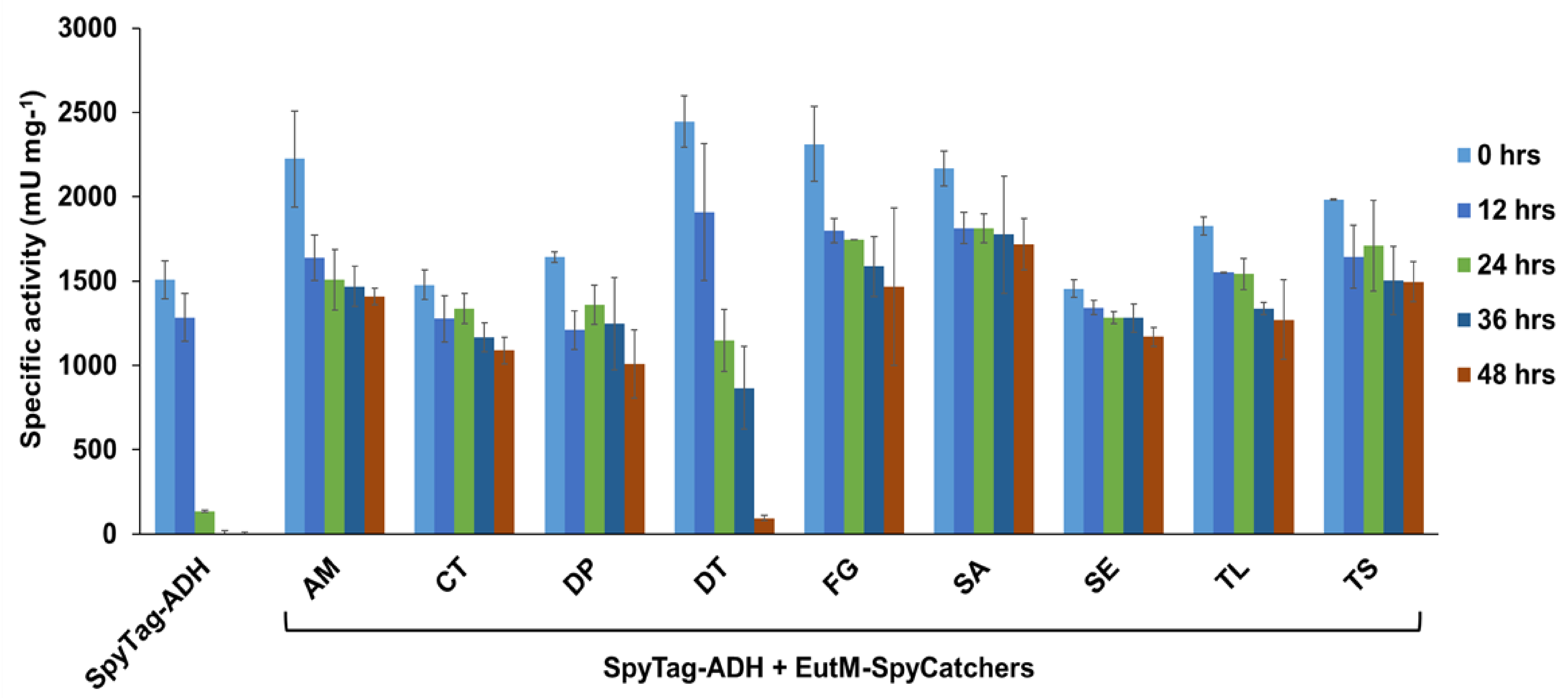


Scaffolds have different effects on ADH activity

Enzyme activities measured with 20 mM (S)-2-hexanol (20 mM), 1 mM NAD+ in 50 mM Tris-HCl (pH 8.0) of ADH (0.2 mg mL^{-1}) in the absence (control) and presence of EutM-SpyCatcher scaffolds (at a 1:9 molar ratio).

ACS Synth Biol (2019) 8:1867-1876.

Enzyme immobilization on EutM-SpyCatcher scaffolds



Enzyme (0.02 mg mL^{-1}) mixed at a 1:9 molar ratio with EutM-SpyCatcher scaffolds in 50 mM Tris-HCl (pH 8.0) and incubated at 30°C for 0-48 hrs.

ACS Synth Biol (2019) 8:1867-1876.

Co-Factor stability & recycling

- Co-factor stability and recycling major limitiation for the production of low- and medium-cost chemicals using biocatalysis due to the high cost of co-factors, especially nicotinamide adenine dinucleotide (NAD(P)) used for commonly used redox reactions in biocatalysis.

- Solutions to date – *In situ* co-factor generation systems and redox neutral and/or co-factor recycling enzyme cascades

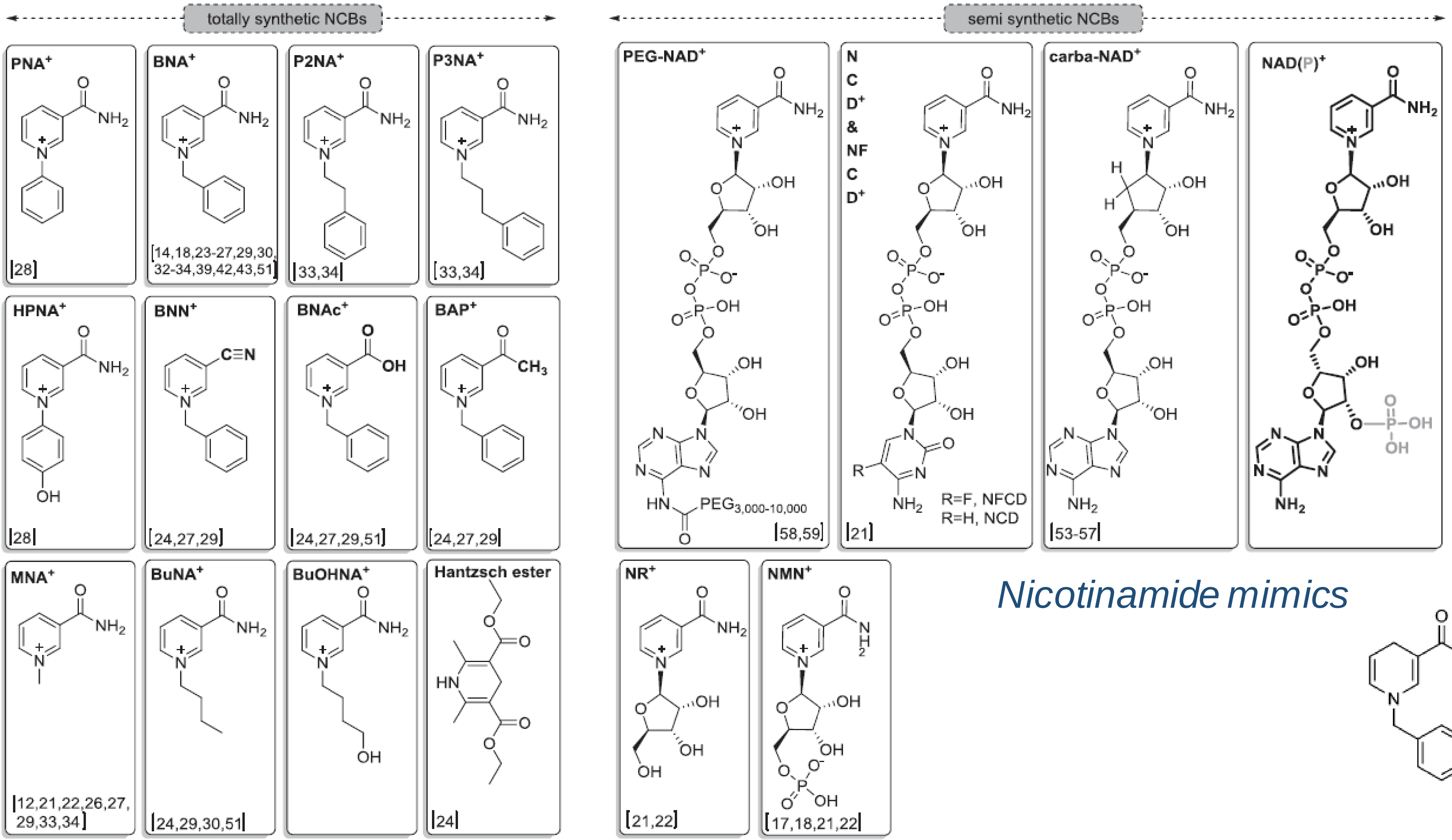
- Co-factor stability problem remains ....

Development of (acid-) stable, synthetic biomimetic co-factors to reduce process costs

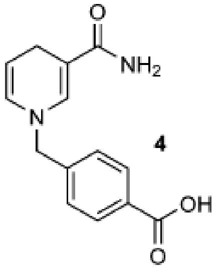
Curr Opin Chem Biol (2019) 49:59-66,

ChemBiochem (2017) 18:1944-1949

Co-Factor stability & recycling



Nicotinamide mimics



4

ChemBioChem 2019, 20, 838 – 845

Curr Opin Chem Biol (2019) 49:59-66,

Synthesis of many biomimetics in 1-2 steps

Co-Factor stability & recycling

FAD-dependent enzyme

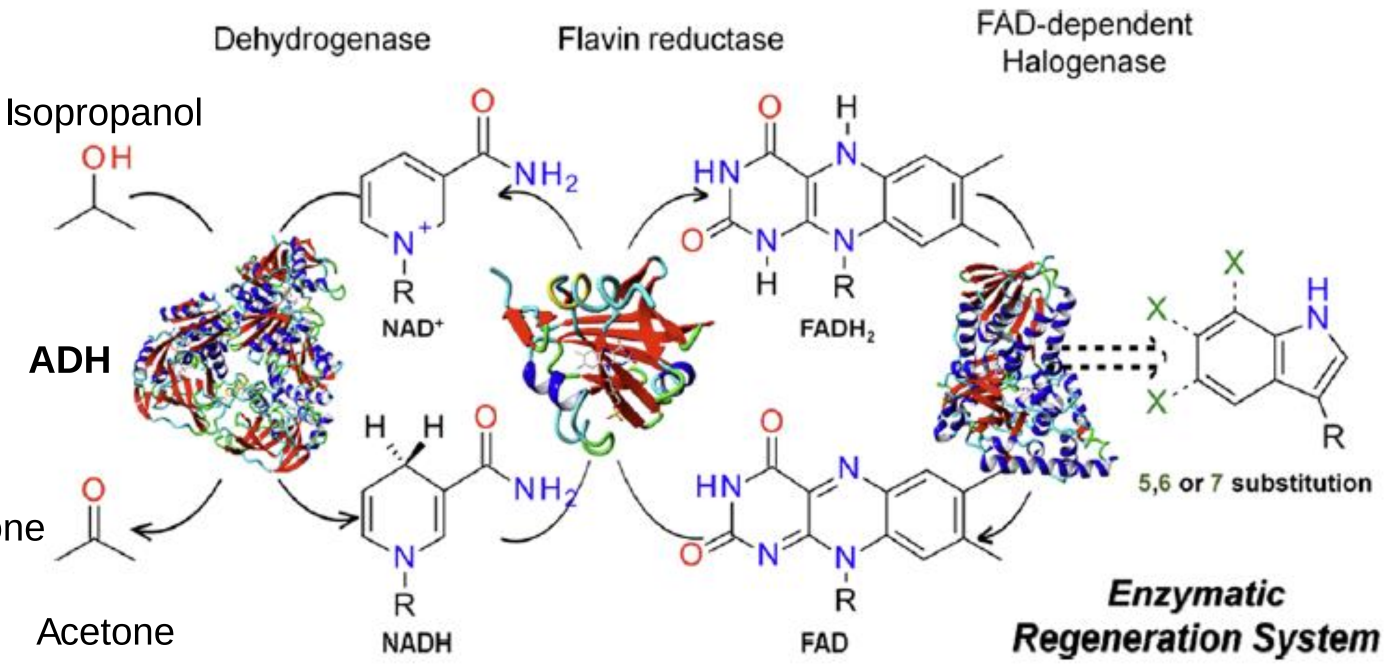
Formate **Glucose**

NAD(P)

Formate DH **Glucose DH**

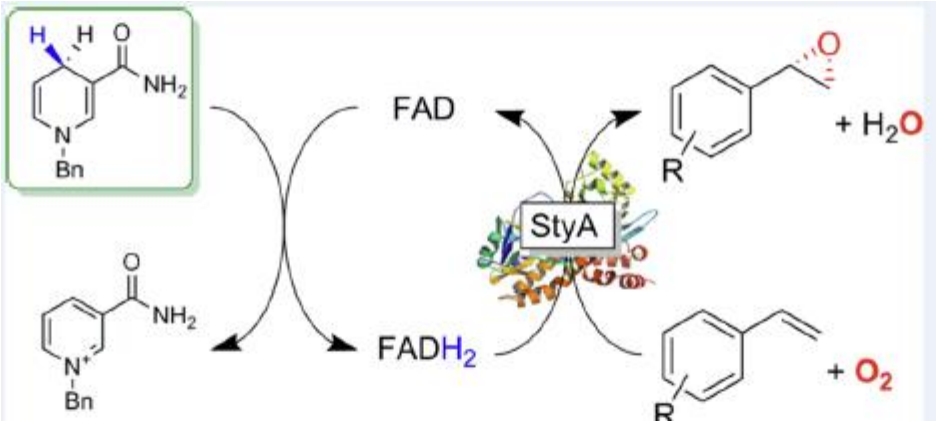
NAD(P)H

CO_2 Glucono1,5-lactone

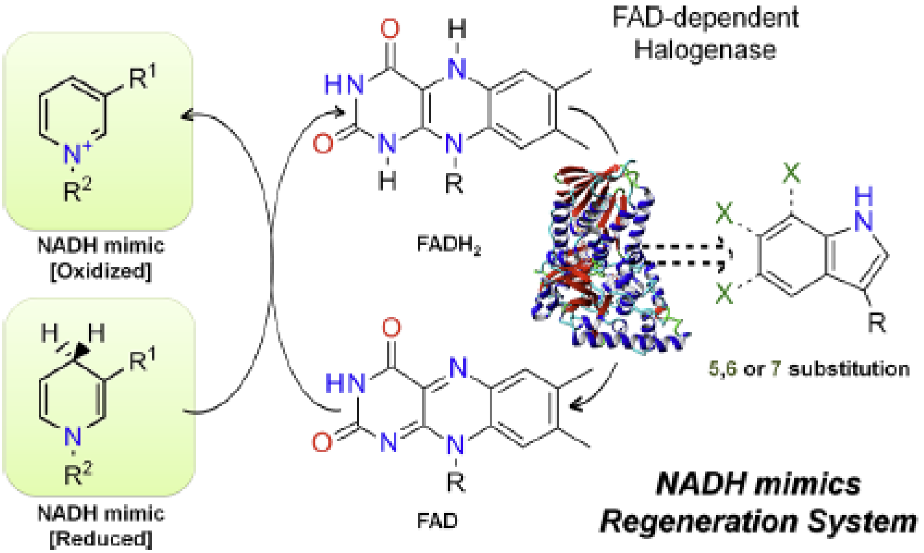


ACS Catal. 2019, 9, 1389−1395

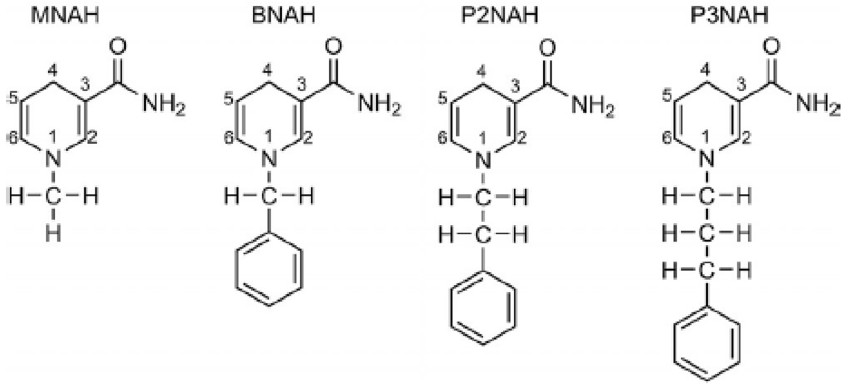
Co-Factor stability & recycling



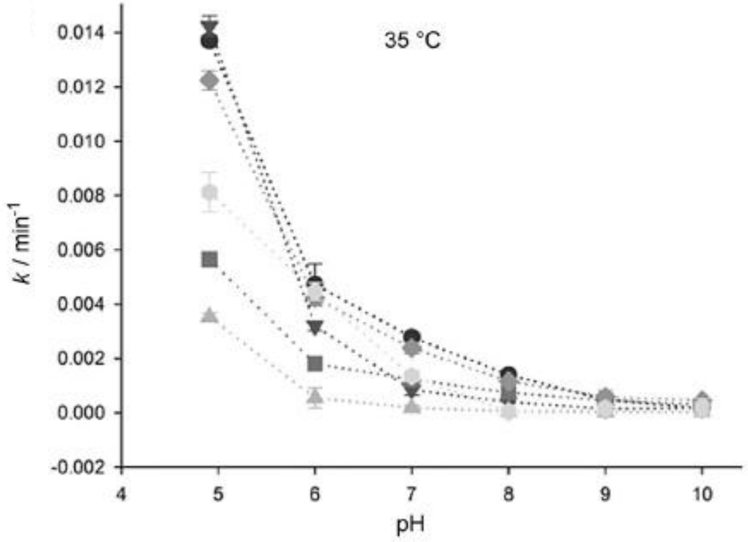
ACS Catal. 2015, 5, 2961−2965

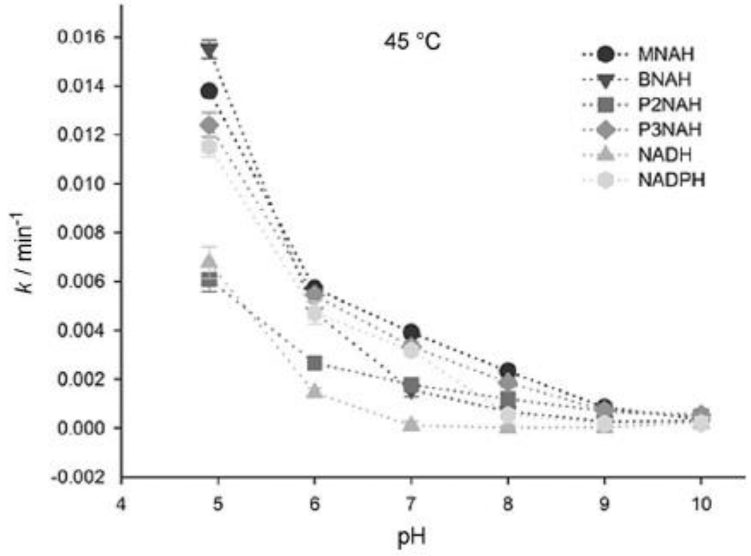


ACS Catal. 2019, 9, 1389−1395



Co-Factor stability & recycling





ChemBioChem 2017, 18, 1944 – 1949

Mimics are not more stable, but cheaper to synthesize

Increasing number of examples show that mimics can yield comparable kinetics compared to natural NAD-cofactors

Limitations so far, lack of enzymatic in situ regeneration system for mimics & general applicability for oxidoreductases

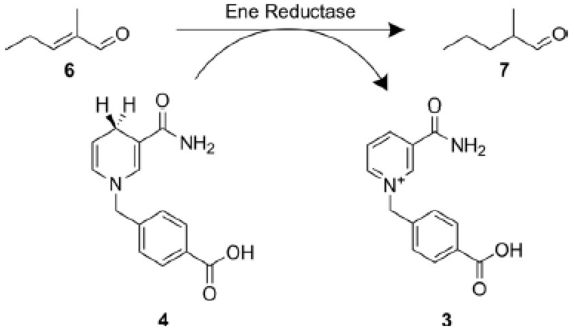
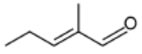
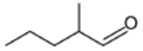
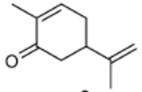
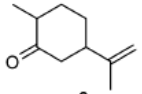
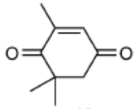
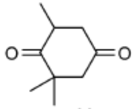


Table 2. The substrates and products used in ER-catalyzed reduction with compound 4 or NADH as the coenzyme, and the kinetic data for each substrate.

		Compound 4			NADH		
Substrate	Product	K_m [mM]	V_{max} [mU]	k_{cat}/K_m [min^{-1}]	K_m [mM]	V_{max} [mU]	k_{cat}/K_m [min^{-1}]
 6	 7	0.07 ± 0.016	15 ± 0.04	111.36	0.03 ± 0.0094	0.8 ± 0.05	11.62
 8	 9	0.22 ± 0.067	4 ± 0.6	7.97	0.36 ± 0.0503	0.9 ± 0.04	1.22
 10	 11	0.26 ± 0.046	3 ± 0.07	5.32	0.48 ± 0.299	0.6 ± 0.04	0.61

K_m: Michaelis–Menten constant, k_{cat}: turnover number [mM min^{-1}] not shown.

ChemBioChem 2019, 20, 838 – 845

Energy & substrate input

Electrons scavenged from glucose, formate, alcohols can drive biocatalytic reduction reactions

$$HCOOH \xrightarrow[NAD(P)^+ \;\curvearrowright\; NAD(P)H]{FDH} CO_2 + H_2O$$

Example – Formate as electron donor

Formate: an Energy Storage and Transport Bridge between Carbon Dioxide and a Formate Fuel Cell in a Single Device

Tracy Vo, Krutarth Purohit, Christopher Nguyen, Brenna Biggs, Salvador Mayoral, and John L. Haan*[a]

ChemSusChem 2015, 8, 3853 – 3858

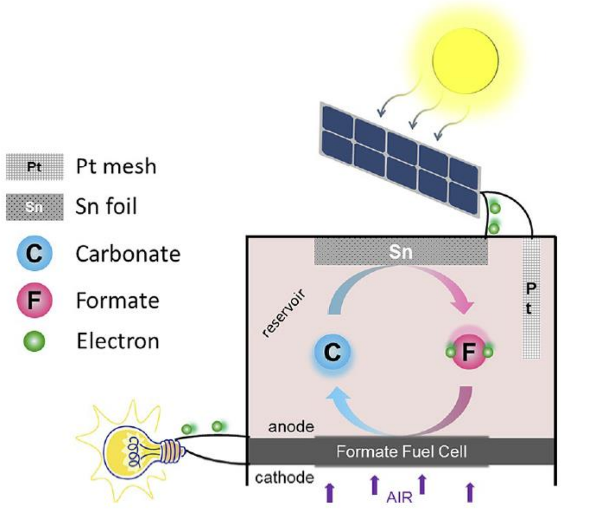
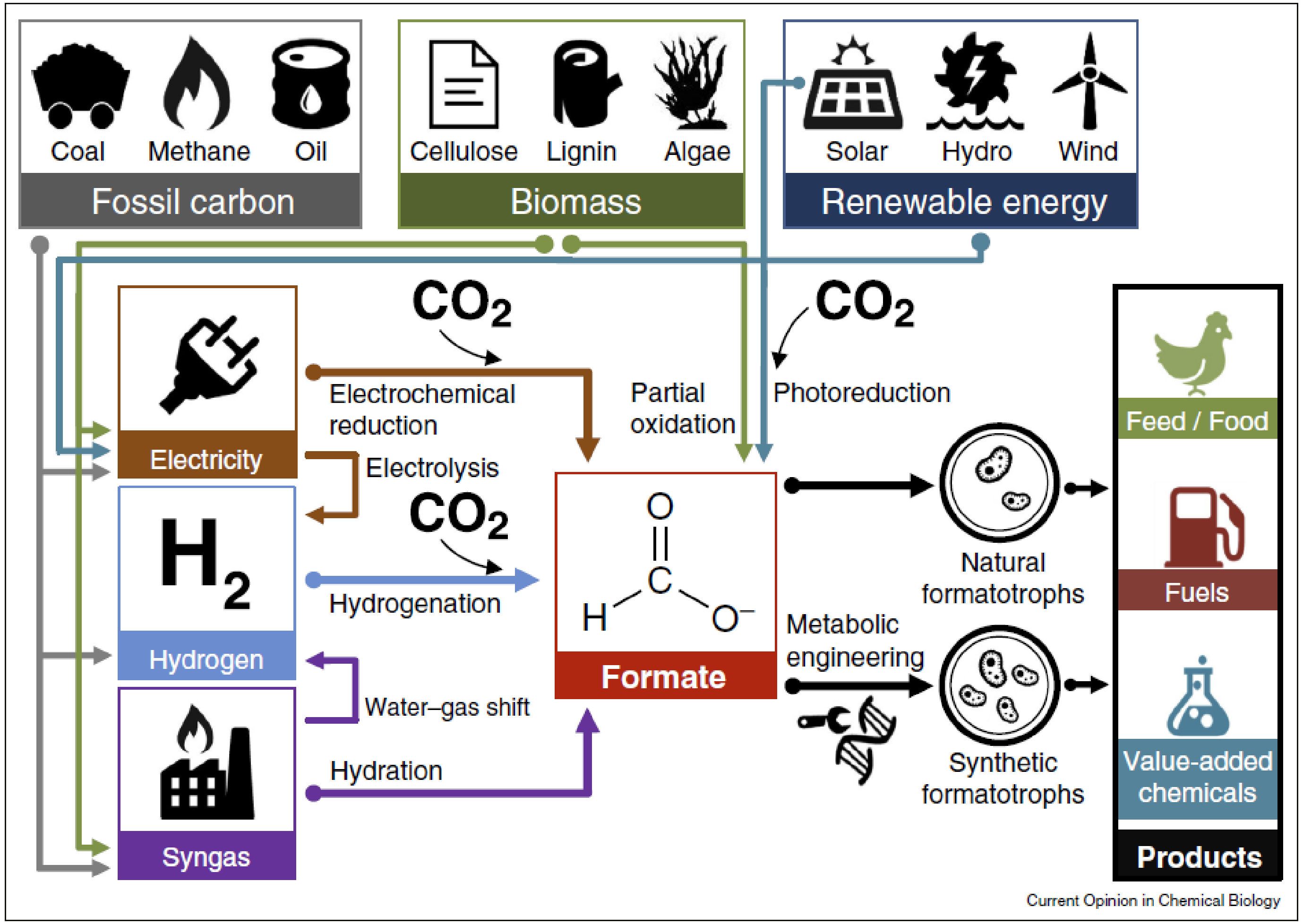


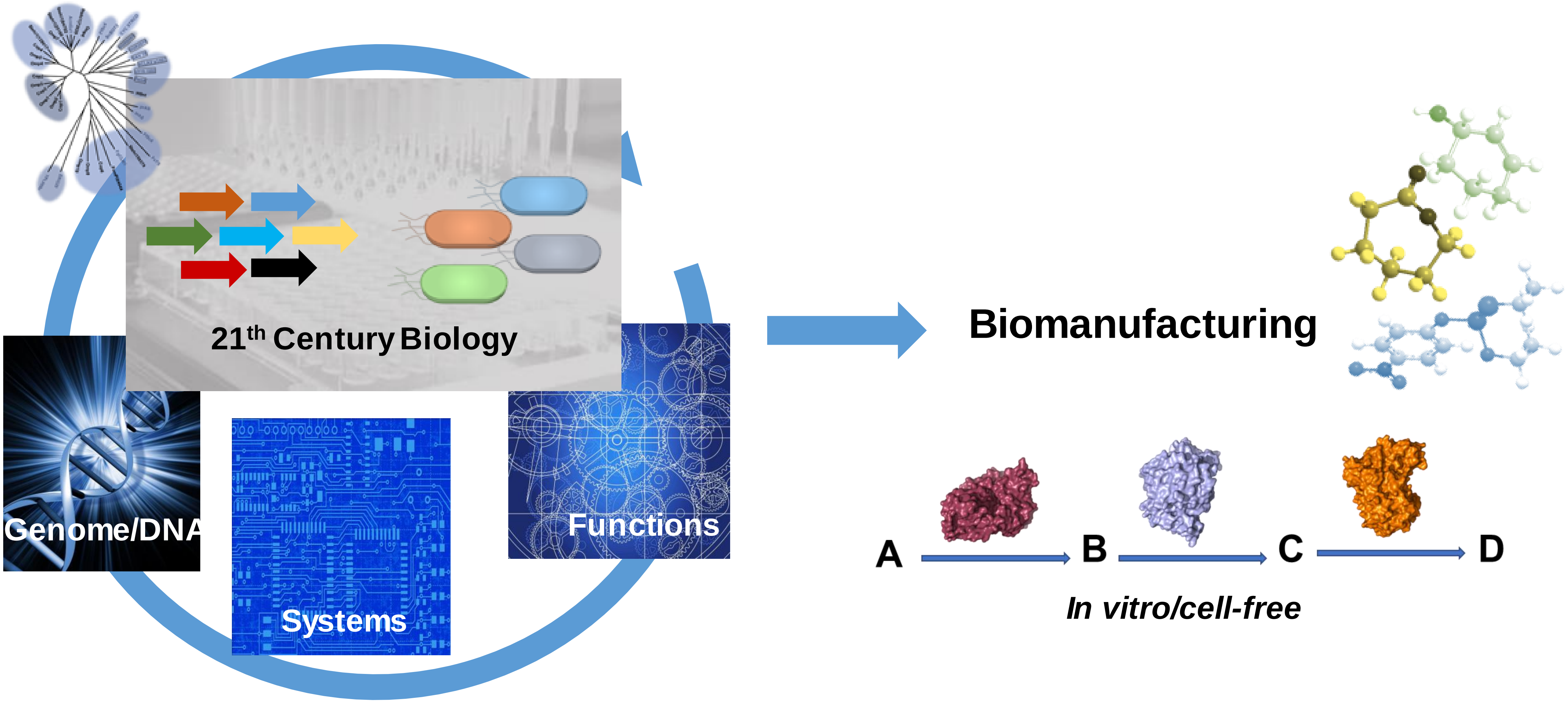
Figure 6. A 2D working principle of the device. Carbonate is converted to formate at a Sn electrode powered by a solar panel. The formate then "carries" the electrons to the DFFC where the formate is oxidized to release the electrons that perform external work. The oxidized formate diffuses back to the reservoir bulk to complete the cycle. Formate can be produced anytime energy is applied to the device, and power can be extracted anytime a load is applied.

Energy & substrate input

The formate bio-economy
Oren Yishai, Steffen N Lindner, Jorge Gonzalez de la Cruz, Hezi Tenenboim and Arren Bar-Even

Current Opinion in Chemical Biology 2016, 35:1–9




21th Century Biology
Genome/DNA
Systems
Functions
Biomanufacturing
A
B
C
D
In vitro/cell-free